Motility and Morphology of Sperm of the Ayu, 
Plecoglossus altivelis, at Different Salinities

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Teleost spermatozoa are immotile in the testis and
often in the seminal plasma. Changes in physical or
chemical factors influencing sperm at spawning, such
as a decrease in osmolality in cyprinids (Morisawa et
al., 1983a), an increase in osmolality in many marine
fishes (Morisawa and Suzuki, 1980; Morisawa, 1985;
Utsugi, unpublished) and a decrease in concentra-
tion of surrounding potassium ions in salmonids
(Morisawa et al., 1983b), initiate sperm movement.
The effects of ion concentration and osmolarity on
sperm motility are not only of biological interest, but
are also commercially important for seed production
in aquaculture. Spermatozoa lose their potential for
motility after movement, and prior to that, become
impotent under unfavorable conditions such as ex-
treme hyperosmolality. Swelling or rupturing of
sperm cells due to hyposmolality is a cause of sperm
impotency in the carp, Cyprinus carpio, and rainbow
tROUT, Oncorhynchus mykiss (Morisawa et al., 1983b;
Billard, 1983). But sperm also become impotent
under hyper- and isotonic conditions. In this study,
the effects of sodium and potassium ions on sperm
motility in the ayu, Plecoglossus altivelis, an amphidr-
omous salmoniform species (Plecoglossidae) were
examined, and sperm morphology in different salini-
ties observed with an electron microscope.

Materials and Methods

Semen from five mature males of Plecoglossus
altivelis obtained from a fish farm in Ozuchi, Iwate
Prefecture, Japan, were collected by gently pressing
the abdomen. Samples of ice-cooled semen were
taken up on the point of a needle, and diluted and
well stirred in about 5 µl of graded sodium and/or
potassium chloride solution on a slide glass at con-
centration intervals of 25 mM, starting from 25 mM.
The activity, duration of motility (motility time) and
percentage of motile spermatozoa were scored and
measured under a light microscope. Sperm activities
were scored immediately after dilution and classified
as follows: + + , very active (rapid movement); + ,
active (not very rapid); ± , vibratory (almost still);
− , inactive (completely immotile). Sperm motility
time was expressed as the duration in seconds from
dilution to the cessation of movement in 95% of the
sample. All measurements and scores were made on
more than ten samples from each individual fish, and
averaged. To investigate the influence of changes in
chemical factors on the sperm, each semen sample,
on which the effects of the NaCl or KCl solutions
had been examined, was diluted again with the alter-
nate solution of equal osmolality and volume to the
initial solution, and sperm response measured and
scored as above. For electron microscopic observa-
tions, semen samples, which were diluted to about
1% and well stirred in NaCl and KCl solutions at
several osmolarities, and an undiluted control semen
sample were held in test tubes for about 5 minutes at
room temperature. Subsequently, they were centri-
fuged at 800 rpm for 5 minutes, and fixed with 2%
gluteraldehyde buffered at pH 7.3 with 0.1 M sodium
cacodylate, for 1 h at 4°C. After further centrifuga-
tion, they were post-fixed with 1% osmium tetroxide,
using the same buffer as above for 1 h at 4°C, dehy-
drated in an ethanol series and embedded in epoxy
resin. Silver or silver-gold sections were made with
glass knives on an LKB Ultrotome, stained with
uranyl acetate and lead citrate, and observed with a
JEOL 100s electron microscope.

Results

Effects of NaCl on sperm motility (Fig. 1).—Most
of the ayu sperm (ca. 90%) was very active (+ +)
in NaCl solution at concentrations from 25 to 175
mM, with little difference in motility time overall
(ca. 30–50 seconds). Clearly, sodium chloride, as a
chemical factor, had little effect on ayu sperm motil-
ity. Immotility of the sperm in high NaCl concentra-
tions (more than 200 mM) and a slight shortening of
motility time in low concentrations (25–50 mM)
were observed, probably caused by hyper- and hyp-
osmolality.

Sperm samples previously diluted and kept in
NaCl solutions for more than 5 minutes were no
longer motile upon additional dilution with KCl.

Effects of KCl on sperm motility (Fig. 2).—Ayu
K. Utsugi

Fig. 1. Effects of dilution with graded NaCl and additional dilution with graded KCl on sperm activity (top), sperm motility time (top), and percentage of motile sperm (bottom) in the ayu, Plecoglossus altivelis.

Fig. 2. Effects of dilution with graded KCl and additional dilution with graded NaCl on sperm activity (top), sperm motility time (top), and percentage of motile sperm (bottom) in the ayu, Plecoglossus altivelis.
sperm was motile in low KCl concentrations (25-75 mM) for twice as long (ca. 50-100 seconds) as in NaCl at the same concentrations, the degree of sperm activity being rated +. 80-90% motility occurred in 25-50 mM KCl solutions, compared with 30% motility in 75 mM KCl, although the motility time in the latter was similar to that in 50 mM KCl. In high KCl concentrations (>100 mM), motility was very low, from 100 to 175 mM KCl, the retention of motility potential was demonstrated by additional dilution with NaCl at the same concentrations. As a result, 80-90% of the sperm became very active (+ +) for about 70 seconds in KCl + NaCl concentrations from 75 to 125 mM. Although motility percentages and times declined in 150 mM KCl (50% motile for ca. 50 seconds) and 175 mM KCl (25% motile for about 50 seconds), sperm became active (+) upon further dilution with NaCl. Sperm samples initially diluted in extremely high concentrations of KCl (more than 200 mM) were no longer motile upon additional dilution with NaCl.

For ayu sperm, the presence of potassium ions in low concentrations (25-50 mM) seemed to prolong sperm motility time and at higher concentrations (100-150 mM), preserve the potential for sperm motility.

**Morphology of ayu sperm under different salinity levels.**—The fixative used in this study appeared effective, although the osmolarity of the fixative (ca. 400 mOsm/l) was somewhat higher than that of the seminal plasma of the species (ca. 300 mOsm/kg—determined with a freezing-point depression osmometer).

Control ayu spermatozoa showed the following morphological features: head with a U-shaped nucleus, a mitochondrion attached to the posterior end of the nucleus (Fig. 3A); mitochondrion crescentic in cross section (Fig. 3B).

The morphology of spermatozoa which had ceased movement in 150 mM NaCl differed considerably
from the control specimens, their mitochondria being clearly swollen (Fig. 3C, D), despite the osmotic environment being similar to that of the control spermatozoa in seminal plasma. Mitochondrial migration to the anterior portion of the nucleus, supposedly by flagellar beating, was often observed in 150 mM NaCl solution (Fig. 4A).

Immotile spermatozoa which had retained their motility potential in 100 and 125 mM KCl were morphologically similar to the control spermatozoa. In 150 mM KCl, however, swollen mitochondria were detected in 60% of spermatozoa (counted from ultra-thin sections).

Spermatozoa which had ceased moving in low concentrations (25-50 mM) of both NaCl and KCl solutions, were found to have ruptured (Fig. 4B). Conversely, in a high concentration (300 mM) of both NaCl and KCl in which sperm died without movement, although mitochondria were found to be swollen, the plasma membrane of the sperm cells was undamaged (Fig. 4C).

The conditions of sperm cells and mitochondria

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**Table 1.** Morphological states of sperm cells and mitochondria of the ayu, *Plecoglossus altivelis*, diluted in different saline solutions for about 5 minutes

<table>
<thead>
<tr>
<th>Saline conc. (mM)</th>
<th>Osmolarity (mOsm/l)</th>
<th>State of sperm cells</th>
<th>State of mitochondria</th>
<th>Motility potential*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>300</td>
<td>normal</td>
<td>compressed</td>
<td>+</td>
</tr>
<tr>
<td>50 NaCl</td>
<td>100</td>
<td>injured</td>
<td>swollen</td>
<td>-</td>
</tr>
<tr>
<td>50 KCl</td>
<td>100</td>
<td>injured</td>
<td>swollen</td>
<td>-</td>
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<tr>
<td>125 NaCl</td>
<td>250</td>
<td>normal</td>
<td>swollen</td>
<td>-</td>
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<tr>
<td>125 KCl</td>
<td>250</td>
<td>normal</td>
<td>compressed</td>
<td>+</td>
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<tr>
<td>150 NaCl</td>
<td>300</td>
<td>normal</td>
<td>swollen</td>
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<tr>
<td>150 KCl</td>
<td>300</td>
<td>normal</td>
<td>compressed or swollen</td>
<td>+</td>
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<tr>
<td>300 NaCl</td>
<td>600</td>
<td>normal</td>
<td>swollen</td>
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<tr>
<td>300 KCl</td>
<td>600</td>
<td>normal</td>
<td>swollen</td>
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</tbody>
</table>

* Motility potential of sperm in each medium is given by + or −.
under several saline conditions are shown in Table 1.

Discussion

Salmonid spermatozoa are able to move in 0-200 mM NaCl and/or 0-400 mM sugar solutions, but are never motile in solutions containing several mM KCl and/or in hypertonic solutions of more than 400 mOsm/kg independent of its components (Morisawa et al., 1983b). Considering fishes generally, only in salmonids are potassium ions known to suppress the onset of sperm movement. On the contrary, the initiation of sperm motility in cyprinids and many marine fishes depend only upon changes in osmolality of the surrounding medium (Morisawa and Suzuki, 1980; Utsugi, unpublished). In fact, the presence of potassium ions is somewhat favorable for sperm motility in cyprinids (Morisawa et al., 1983a). In the ayu, sperm motility patterns resembled those of salmonids, an anadromous group (motility time <1 minute, immotile in >200 mM NaCl, motility suppressed by potassium), except in the degree of suppression of sperm motility by potassium ions (>100 mM in Plecoglossus altivelis vs. several mM in salmonids). However, promotion of motility by potassium, as seen in cyprinids, was observed.

The physiological nature of ayu sperm, such as low sensitivity to potassium, permits successful spawning downstream and at river mouths, where potassium (from sea water) exists in high concentrations, and suggests a close relationship of the species with other anadromous species such as the Osmeridae, which include sea-spawning species (e.g. Hypomesus pretiosus). It is considered that other similarities in sperm motility patterns between the ayu and salmonids reflect their phyletic closeness.

Promotive effects of potassium on sperm motility are likely to be common in fishes, because the sperm motilities of many sea-spawning species are low in potassium-free media (Utsugi, unpublished). Furthermore, incapacitated sperm of marine-captured chum salmon, Oncorhynchus keta, were capacitated and their motility accelerated by dipping sperm into isotonic KCl for a few minutes prior to dilution for the purpose of sperm activation (Utsugi, unpublished). Accordingly, it may be said that the suppressive effects of potassium on the initiation of sperm motility is specific in salmoniform fishes among teleosts.

In the present study, all of the spermatozoa which had lost their motility potential had swollen mitochondria (Table 1). Even in 150 mM KCl, 60% of spermatozoa, which were probably directly related to the immotile faction recorded during experimentations, possessed swollen mitochondria. Therefore, swelling of the mitochondria is considered as indicative of the death of the spermatozoa. It is obvious that such swelling was not caused by osmotic factors, because the phenomenon was detected in a wide range of saline concentrations, from isotonic to hypertonic, leading to the conclusion that impotency of spermatozoa in Plecoglossus altivelis under any conditions is caused primarily by disfunction of the mitochondria. Osmotic injuries of sperm cells such as swelling and rupturing in hypotonic conditions, rather than being direct factors causing sperm impotency, should be considered as secondary events.

Acknowledgments

I am grateful to Professor Yasuhiko Taki, Tokyo University of Fisheries, for his critical reading of the manuscript. I wish to dedicate this work to the late Dr. Motoyoshi Yokote, who was my technical adviser.

Literature Cited


種々の塩分条件下におけるアユ精子の運動性と形態

打木研三

アユの精子の運動性に対するK⁺, Na⁺, およびそれぞれのイオ
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の浸透濃度の影響を調べた。その結果、アユの精子は塩分組成
とは無関係に 400 mOsm 以上の浸透濃度下では全く運動せず、
潜在的運動能力をも失った。精子の運動を許容する濃度範囲に
おいて、Na⁺ は精子の活性や運動時間に影響を与えないが、
一方 K⁺ は低濃度では僅かに活性を低下させるかわりに運動時
間を延長させ、中濃度では強く運動を抑制するかわりに潜在的
運動能力をむしろ高め、高濃度では強く運動を抑制するととも
に潜在能力をも低下せしめるという、濃度の違いに応じた様々

な影響を与えた。またこれと平行して、透過型電子顕微鏡で諸条
件下における精子の形態観察を行なったところ、潜在的運動能
力を喪失したすべての精子にミトコンドリアの著しい膨潤が観
察されたことから、精子の死はミトコンドリアの機能不全によ
るものと判断された。

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